JOURNAL OF BACTERIOLOGY, July 1995, p. 3824–3829 Vol. 177, No. 13
0021-9193/95/$04.00+0

Phage Operon Involved in Sensitivity to the *Lactococcus lactis* Abortive Infection Mechanism AbiD1

ELENA BIDNENKO, DUSKO EHRLICH, AND MARIE-CHRISTINE CHOPIN*

Laboratoire de Génétique Microbienne, Institut National de la Recherche Agronomique, 78352 Jouy-en-Josas, France

Received 12 December 1994/Accepted 14 April 1995

Phage bIL66 is unable to grow on *Lactococcus lactis* cells harboring the abortive infection gene *abiD1*. Spontaneous phage mutants able to grow on AbiD1 cells were used to study phage-Abi interaction. A 1.33-kb DNA segment of a mutant phage allowed growth of AbiD1^s phages in AbiD1 cells when present in *trans*. Sequence analysis of this segment revealed an operon composed of four open reading frames, designated *orf1* to *orf4*. The operon is transcribed 10 min after infection from a promoter presenting an extended −10 consensus sequence but no −35 sequence. Analysis of four independent AbiD1^r mutants revealed a different point mutation localized in *orf1*, implying that this open reading frame is needed for sensitivity to AbiD1. However, the sensitivity is partly suppressed when *orf3* is expressed in *trans* on a high-copy-number plasmid, suggesting that AbiD1 acts by decreasing the concentration of an available *orf3* product.

The growth of a lytic bacteriophage can be arrested by several bacterial phage defense mechanisms (8). One of them is characterized by a normal start of phage development followed by an abrupt interruption, resulting in the release of few or no progeny particles and cell death. Such abortive infections (Abi) were first described for *Escherichia coli* and *Bacillus subtilis* and have been identified more recently in *Lactococcus lactis*. The molecular basis of many of these phage exclusion systems remains unclear. Understanding progressed recently for three of them (28). In every case, phage infection induced deleterious effects for highly conserved cellular components involved either in the translation apparatus or in membrane potential. This results in cell death and phage growth arrest, which blocks the spread of phages to other cells.

Four lactococcal genes coding for an Abi phenotype have been cloned and sequenced (2, 6, 7, 9, 15). Except for AbiA (16, 23), which has been proposed to interfere with phage DNA replication, the molecular mechanisms of lactococcal phage growth impairment in Abi cells are unknown. AbiD1 is active on two lactococcal phage species, referred to as the 936 and C6A phage groups, respectively (18). The study of phage multiplication in AbiD1 cells could therefore provide insight into both the molecular basis of phage-Abi interactions and the relationship between the two main lactococcal phage species.

The spontaneous appearance of mutants of the small-isometric-headed phage bIL66, able to grow on AbiD1 cells, has been observed (11). We report that a 1.33-kb segment from a mutant phage allows the development of sensitive phages when present in AbiD1 cells.

Sequence analysis of this segment revealed four putative open reading frames (ORFs) which form an operon. The operon is transcribed 10 min after infection. Two of these ORFs play a role in phage-Abi interaction. Point mutations in *orf1* confer AbiD1^r, but the *orf3* product is required for the growth of sensitive phages. We propose that the *orf1* and *abiD1* gene products interact to prevent translation of the *orf3* RNA. The amount of *orf3* product in the infected cells is thus decreased, which prevents phage multiplication.

* Corresponding author. Phone: 33 1 34 65 25 30. Fax: 33 1 34 65 25 21.

MATERIALS AND METHODS

Bacterial strains, plasmids, phages, and media. *L. lactis* subsp. *lactis* IL1403 (5) and derivatives were grown at 30°C in M17 medium (30), in which lactose has been replaced by glucose (M17glc). *E. coli* TG1 (12) and *B. subtilis* MT119 (29) were grown at 37°C in Luria-Bertani medium (22). Erythromycin (0.3 μg/ml for *B. subtilis*, 5 μg/ml for *L. lactis*, and 150 μg/ml for *E. coli*) or ampicillin (50 μg/ml) was added to the culture medium when needed. Plasmid pIL105, which confers the AbiD1 phenotype, was from the wild-type *L. lactis* subsp. *cremoris* strain IL964 (11). Phages bIL66, bIL67, bIL170, and bIL188 were from our laboratory collection and were enumerated as described before (30). Phages bIL66 and bIL170 belong to the 936 phage group, and phage bIL67 belongs to the C6A phage group (2). Phage 188 was shown to belong to the C6A phage group by DNA hybridization with the type phage P001. Phages P001 and phivML3 were kindly provided by M. Teuber and M. Gasson, respectively.

Molecular cloning and DNA sequence analysis. The procedures for DNA manipulation, transformation of *E. coli* cells, and cloning were essentially as described by Maniatis et al. (22). Electrotransformation of *L. lactis* was carried out as described before (17). Transformation of *B. subtilis* competent cells was done by the method of Anagnostopoulos and Spizizen (1).

E. coli clones for sequencing, sequence determination, and analysis were handled as described in the accompanying paper (2).

mRNA extraction, labelling, and primer extension analysis. Total RNA was extracted from phage-infected cultures of *L. lactis* by the method of Glatron and Rapoport (13) as modified by R. Raya (unpublished data). Northern (RNA blot) experiments were carried out essentially as described by Williams and Mason (33). Oligonucleotides used as probes, complementary to the sequence shown in Fig. 2, were as follows: 1, 5′GGAGGTAACTGTGGCTAGAG3′ (positions 356 to 337); 2, 5′CAAACCACATTCAACAGCG3′ (positions 566 to 548, *orf1*); 3, 5′GGTACTATGTTATAGCTC3′ (positions 1127 to 1144, *orf3*); 4, 5′GGGCT GTTAGTTGGCGACAC3′ (positions 1500 to 1481); 5, 5′CTTGGACTGGAC CTGCAC3′ (positions 1699 to 1681); 6, 5′GCTATACGTGGACTGATGG3′ (positions 2065 to 2047); 7, 5′GGGGCGGGTACCTATGGG3′ (positions 2208 to 2191); and 8, 5′CACGCTCATACGAGCCC3′ (positions 2388 to 2372).

The 5′ ends of mRNA were determined by the reverse transcriptase-directed primer extension method (33), modified as described in the accompanying paper (2). The synthetic oligonucleotide (5′CAAACCACATTCAAG3′) was complementary to coordinates 546 to 566 of the sequence shown in Fig. 2.

Nucleotide sequence accession number. The sequence described in this paper has been assigned GenBank accession number L35175.

RESULTS

Characterization of phages bIL66 and bIL66M1. bIL66 is a small-isometric-headed lactococcal phage belonging to the 936 phage group (18). Restriction analysis indicated that its genome is linear and carries cohesive ends (data not shown). A restriction map of the phage was constructed (Fig. 1). bIL66 forms large clear plaques on strain IL1403 and small turbid plaques at an efficiency of 10^{-4} on IL1403(pIL105) (Table 1).

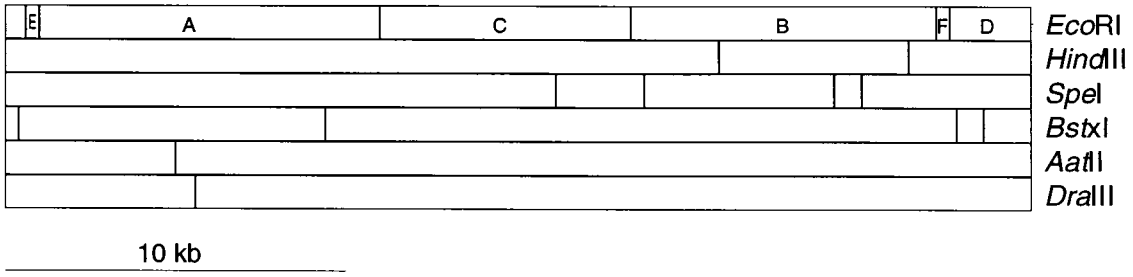

FIG. 1. Restriction map of phage bIL66.

Spontaneous mutants that form clear plaques on the latter strain and are therefore resistant to AbiD1 appear at a frequency of about 10^{-5} (Table 1). One of the clear-plaque mutants was purified and designated bIL66M1. No difference in the restriction maps of bIL66 and bIL66M1 was observed (data not shown), indicating that neither a large deletion nor a DNA rearrangement had occurred in bIL66M1. The phage forms clear plaques with a 10-fold-lower efficiency on IL1403 (pIL105) than on IL1403 (Table 1), indicating that the mutation partially suppresses the AbiD1 phenotype.

Identification of a phage bIL66M1 DNA segment conferring resistance to AbiD1. We reasoned that a segment of bIL66M1 bearing the mutation might allow growth of AbiD1^s phages in IL1403 AbiD1 when supplied in *trans*, either by complementation or by recombination. To test this hypothesis, *Eco*RI segments of phage bIL66M1 DNA were cloned into the plasmid vector pBluescript (Stratagene, La Jolla, Calif.) in *E. coli* and transferred into the high-copy-number *B. subtilis*/*L. lactis* vector pIL253 (27). Four of the six segments were successfully cloned. One segment (C) (Fig. 1), unstable in *E. coli*, was cloned directly into pIL253 in *B. subtilis*. Cloning of the last segment (B) (Fig. 1) failed in three different hosts (*B. subtilis*, *E. coli*, and *L. lactis*), possibly because of its structural instability or toxicity. Matching segments from the wild-type phage were cloned as a control (segment B was not obtained). None of the cloned segments changed the sensitivity of IL1403 to phages.

The five cloned segments were introduced into IL1403 AbiD1 on pIL253. In the presence of segment D, clear plaques were observed for the prolate-headed phages bIL67 and bIL188, and the number of clear plaques formed by bIL66 was increased 200-fold. In addition, the number of turbid plaques formed by the isometric-headed phage bIL170 and the two prolate-headed phages was increased between 10^2- and 10^5-fold (Table 1). This stimulation of phage development was not observed in the presence of the corresponding segment from the wild-type phage or any of the other segments of the mutant or wild-type phage (not shown). The mutated segment D had no effect on phage bIL66M1 (Table 1). This segment is 3.1 kb long and overlaps the phage cohesive ends (Fig. 1). pIL253 carrying segment D of phage bIL66M1 was designated pIL1912.

TABLE 1. Phage development in cells harboring the AbiD determinant

Phage	Phage titer (plaques/ml) in strain[a]:		
	IL1403	IL1403(pIL105)	IL1403(pIL105, pIL1912)
Isometric			
bIL66	1×10^9	1×10^4, 1×10^5 T	2.6×10^6, 1×10^4 T
bIL66M1	5×10^8	4×10^7	4×10^7
bIL170	5×10^8	<1	1×10^5 T
Prolate			
bIL67	1×10^{10}	1×10^4 T	8×10^4, 1×10^6 T
bIL188	1×10^{10}	1×10^4 T	1×10^5, 1×10^7 T

[a] pIL1912 carries the *Eco*RI segment D (Fig. 1) of phage bIL66M1. T, small turbid plaques, very difficult to count; the values are only indicative.

To test whether the increased number of plaques resulted from a diffusible gene product specified by segment D or from recombination between the infecting phage and the cloned segment, phages were picked up from 20 independent plaques formed by bIL170 on IL1403(pIL105, pIL1912), purified, and propagated on IL1403. The lysates obtained were enumerated on IL1403, IL1403(pIL105), and IL1403(pIL105, pIL1912). None of the isolates was resistant to AbiD, and all grew with an efficiency similar to that of the parental phage on cells containing both *abiD1* and segment D of the mutant phage (data not shown). Similar results were obtained with phages bIL67 and bIL188. The development of these phages in IL1403 AbiD1 is therefore the result of the presence of a diffusible gene product encoded by segment D. Interestingly, when this segment was cloned on plasmid pIL252, a low-copy-number homolog of pIL253 (the two plasmids have about 10 and 100 copies, respectively) (27), phage development was not observed, suggesting that it depends on the amount of the diffusible gene product.

A similar analysis was carried out with 100 independent clear plaques formed by bIL66 on IL1403(pIL105, pIL1912). Only three isolates were AbiD1^s, indicating that they resulted from complementation. The others were AbiD1^r and could result either from recombination or from stimulation of the mutation towards resistance.

Subcloning and sequencing of segment D from phage bIL66M1. Subcloning experiments localized the determinant of AbiD1^r on a 2.4-kb segment, which was sequenced (Fig. 2). Part of the sequence (coordinates 2141 to 2400) has 86% identity with the nucleotide sequence of the ends of the genome of the lactococcal phage sk1 (4). The 10-bp sequence corresponding to the cohesive ends of this phage (4) is fully conserved, suggesting that the two phages have the same cohesive ends (boldface in Fig. 2). Four ORFs of 42, 43, 160, and 43 codons preceded by a putative ribosome-binding sequence (31) were detected. *orf1* and *orf2* are separated by 4 bp, whereas *orf2* and *orf3* and *orf3* and *orf4* overlap by 4 and 1 bp, respectively. Since such small ORFs are infrequent, we confirmed their existence by using the Gene Mark program for prediction of genes (3).

The *orf3*-specified protein has 43% identity (59% similarity) with the protein specified by an ORF that is located upstream of the lysin gene on lactococcal prolate-headed phages bIL67 (25) and phivML3 (26). This homology was also observed at

```
CTTTAGAAATCTTACAAACAATAAGCTAATTGTCCTTACTGATACCATACTTTACAAACAGGACACACAATGCACTTACTTTCTGCCACTTCTAGTCAAATTGCGGTCAAGCGTAAAACA   120

AAAGCCCTAAGGGGCTAATTCCTTTTTTAATATAATTTATTTATTTCCTCCTAAGTCAAAGTGTATAGTCGGTTGACTATTCCAAGTATCTAATGGTTTCCTTGTCTACTTCTGGCTGAT   240

TCATATACTCTCTATTCATTCTAACTCTTGTGTTACCTACTTTAATTTTAATACGCTTCTTGTATTCCTGCTGTCGTAAGTACATTAAATATTTATCTCTAGCCACAGTTACCTCCTATA   360

AAGATTATAACACAAAATGCCTACAAAGTCAATCATAGCTTACATAACAGAGGATAAACCAAACCTGAAAAGTGCATTTGTTATAATATATATATCAAGTTAAGAGAGGAAAGCAAATGA   480
                                                                              ---------                  ----       M T
                                                                                -10                       RBS  orf1 --->
CAGAAGAACAGCTACTATTTAAGCAAGAAACATTGTCAATGGTTGACTTTAACGAGTTCTTACTTAACGCTGTTGAATGTGGTTTGATTAATCTTGATACAGATTTAATTTTTAAAGGAG   600
 E  E  Q  L  L  F  K  Q  E  T  L  S  M  V  D  F  N  E  F  L  L  N  A  V  E  C  G  L  I  N  L  D  T  D  L  I  F  K  G  E
                                                                                                                 -------
                                                                                                                   RBS
AATAAAGAAATGAATAAAGAACATATTTTAGCACAAAAAGAAGTATTAACTCCAATTGAGTACGAACACTATGTTAAGCACTTATTTGATATTGGAGAAATTACTAAAGAGCTTTATATT   720
 *      M  N  K  E  H  I  L  A  Q  K  E  V  L  T  P  I  E  Y  E  H  Y  V  K  H  L  F  D  I  G  E  I  T  K  E  L  Y  I
        orf2 --->

GAATTGAGTTCTGATTTATGAGCAAAGCCTTAGCGATTGACTTTAGTACTTCTAATACTGGTTATGCGTTTCGCAATCCTTTAACAAATGAGTATGTAGTCGGTTCAATTGCAGGTGGTA   840
E  L  S  S  D  L  *
                 M  S  K  A  L  A  I  D  F  S  T  S  N  T  G  Y  A  F  R  N  P  L  T  N  E  Y  V  V  G  S  I  A  G  G  K
     ---         orf3 --->
     RBS
AAAGCAAAGACCCTTTGGAACGTGCCAAGATTATAGCTGACGGCATAACAGAAGTCATTGAGCATTATAACTTATTTGATTATTTTATTTATATTGAAGAACCTATTATCACGTTCAAGT   960
 S  K  D  P  L  E  R  A  K  I  I  A  D  G  I  T  E  V  I  E  H  Y  N  L  F  D  Y  F  I  Y  I  E  E  P  I  I  T  F  K  S

CTAAAGGTAATATCTCATTGATTAGAGCTAACGGTTCATTCTTAGGAGTCATGCGTAACCGTCATAATATTGGCTATGTTGATATCCCTAATTCCAAATGGTGCGGTTATCATCTAATTA  1080
 K  G  N  I  S  L  I  R  A  N  G  S  F  L  G  V  M  R  N  R  H  N  I  G  Y  V  D  I  P  N  S  K  W  C  G  Y  H  L  I  K

AAGGTAAGAGTGCATTGCGAAAAGTACAAAGCATTGAGATACTCAAGAGCTATAACATAGTACCTGATAATGATATCAATGATGACCAAGCAGACGCGTTCTGTATCTTGCTCTATGTAG  1200
 G  K  S  A  L  R  K  V  Q  S  I  E  I  L  K  S  Y  N  I  V  P  D  N  D  I  N  D  D  Q  A  D  A  F  C  I  L  T  Y  V  E

AAAGTCAGGAGAATATATAATGATTGTAATTAATATTGCCTTGATTATTCTTGGCATTTTATATGGTGTAGGTTCGGTTACCAACTTTAAGGAGTGGTACTATCGCCATGACTATCTAGC  1320
 S  Q  E  N  I  *
      -----        M  I  V  I  N  I  A  L  I  I  L  G  I  L  Y  G  V  G  S  V  T  N  F  K  E  W  Y  Y  R  H  D  Y  L  A
       RBS         orf4 --->

TATTATGTTAAGTGTATTTACATCCATCTAATTGGTAGTAGCTGGATATAACCGTATTGATTTGAAAGATTAGGTGCCCTGATTGAGGGTGCTTAATTGTTATAGAGTTAACAGCCTAAG  1440
 I  M  L  R  V  F  P  S  I  *

CAATAGGGTGCAAGGTGACGGGAATGCCTTAGTTAAATAAGTGTCGCCAACTAACAGCCCTTTCCAATTAGAGATATAAGTAAGCTGTGATAATAGCCTTGGCTACTTCAAATGGTTCGA  1560

GTCCATTGTATCTCATTCTCCTTTATTTATAATATGTCAGTGAACCTTTAACGTAGCTGGTTTATAATACCACTTGATATAGATAATAGTAAGAGGTAGCGCCTTGAGCTAAGGAATACT  1680

GGTGCAGGTCCAGTCCAAGTGATAGTGGTGTATAGTCCATAGAAGAAGTGCTAAGCAATGGCGCAGTACCTTGGCATAACTATACTTAATCATATTGTGGGCAACTGTGCATGGTTGCTA  1800

AGGTATGAGGTAAGAGTAATTGGAGGAAGTCAAGGTCGCAACTTTTTGGGGTTCGATTCCCTACTCTGCTCTATACTAAGATAGTATCTAATGAGATACCGGCTGCTGATAGTAAGGAAT  1920

AACAAGATGAGGTAGTCATAGTTAGCGGTATAGTCTAATGGTAAACGTGGGTTCGATTCCTACTACTGCTATAAGATAAGGGAGAAACAAATGATTATATTATTATAATTTATAATAATG  2040

TGGTCCATCAGTCCACGTATAGCATTGCTGATATTATTGTAAGCTATTAATCCAGTGTTCATATTGCTATGGCTATTAGTATGGCTTGCAATTAAGCTATAAGCTAAGCAATAAAGTAAT  2160

TGTGAAAAGAAAATAAAATAAAAATTTTCCCATAGGTACCCGCCCCATTAATCGCTATGTTAAGGAGATTTTAAGCACAAAGGACTCCCCTTTACAAATCTCCCAAAAAATCAAAAAGAA  2280

AAGTTTTAGCTAGGTTCAATCTAAATTAATCTATTTTAATCTAGTACGATCTTGCTAAATCTTAAATCATAGGGCTATATGCCCATATTTGGGCTCGTATGAGCGTGTTTATATCATTGC  2400
```

FIG. 2. Nucleotide sequence of the 2.4-kb region of phage bIL66M1. The deduced amino acid sequence is indicated; numbers to the right refer to nucleotides. The potential ribosome-binding sites (RBS) and −10 promoter sequence are underlined, stop codons are indicated by an asterisk, and transcription start and putative cohesive ends are in boldface letters.

the nucleotide level (60% identity along 465 bp, coordinates 738 to 1202 on bIL66M1). A putative ATP-binding site motif A (P-loop) (24, 31) is present in *orf3* (GSIAGGKS). No homology for proteins specified by the other three ORFs was found in the data banks.

***orf3* necessary for complementation of AbiD1^s phages.** Further deletion analysis indicated that a 1.33-kb segment lacking *orf4* and 74 bp from the 3′ end of *orf3* as well as part of the region upstream of *orf1* still allowed complementation of all phages tested. Deletion of the entire *orf3* suppressed complementation, indicating that at least part of this ORF is required. The results obtained with the isometric-headed phage bIL170 are summarized in Fig. 3. Similar results were obtained with the two prolate-headed phages bIL67 and bIL188.

Point mutations in *orf1* confer AbiD1^r on phage bIL66. To localize the mutation present in bIL66M1, we postulated that an infecting phage would recombine at high efficiency with a resident plasmid carrying a homologous phage segment. Marker rescue experiments were carried out. For this purpose, different segments were cloned into pIL253, the plasmids were transformed into IL1403(pIL105), and the cells were infected with wild-type phage bIL66. Clear plaques were formed, as expected. The results, summarized in Fig. 3, indicate that a 72-bp region localized within *orf1* is needed for phage growth. The corresponding region of the wild-type phage was sequenced. Comparison with the phage bIL66M1 sequence revealed only one change, a change of the C at coordinate 583 in the wild-type phage to an A in the mutant (Fig. 2). This results in replacement of an alanine by an aspartic acid. The entire wild-type 1.33-kb segment was also sequenced and found to be identical to that of the mutant.

In order to further define the region in which mutations conferring AbiD1^r occur, three other independent bIL66 mutants were isolated and analyzed. A point mutation affecting

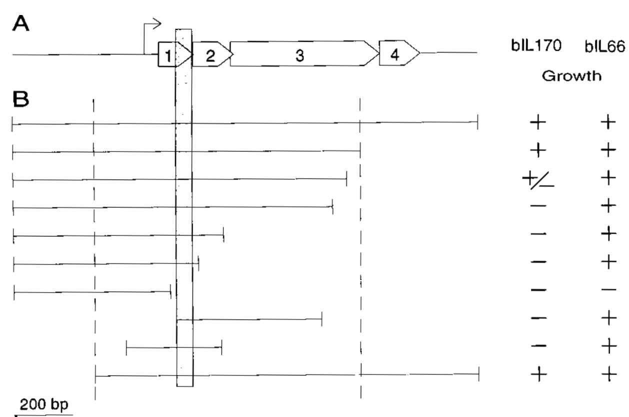

FIG. 3. Deletion analysis of the 1.33-kb segment from phage bIL66M1 conferring AbiD^r. (A) Organization of segment B. bIL170 and bIL66 growth on IL1403 AbiD derivatives harboring different subsegments from bIL66M1 cloned into pIL253. For bIL170: +, titers of 10^5 turbid plaques per ml; −, no plaques; for bIL66: +, 10^9 clear plaques per ml. Growth of phage bIL170 resulted from complementation by the region between dotted lines. Growth of phage bIL66 resulted from recombination with the boxed and shaded region.

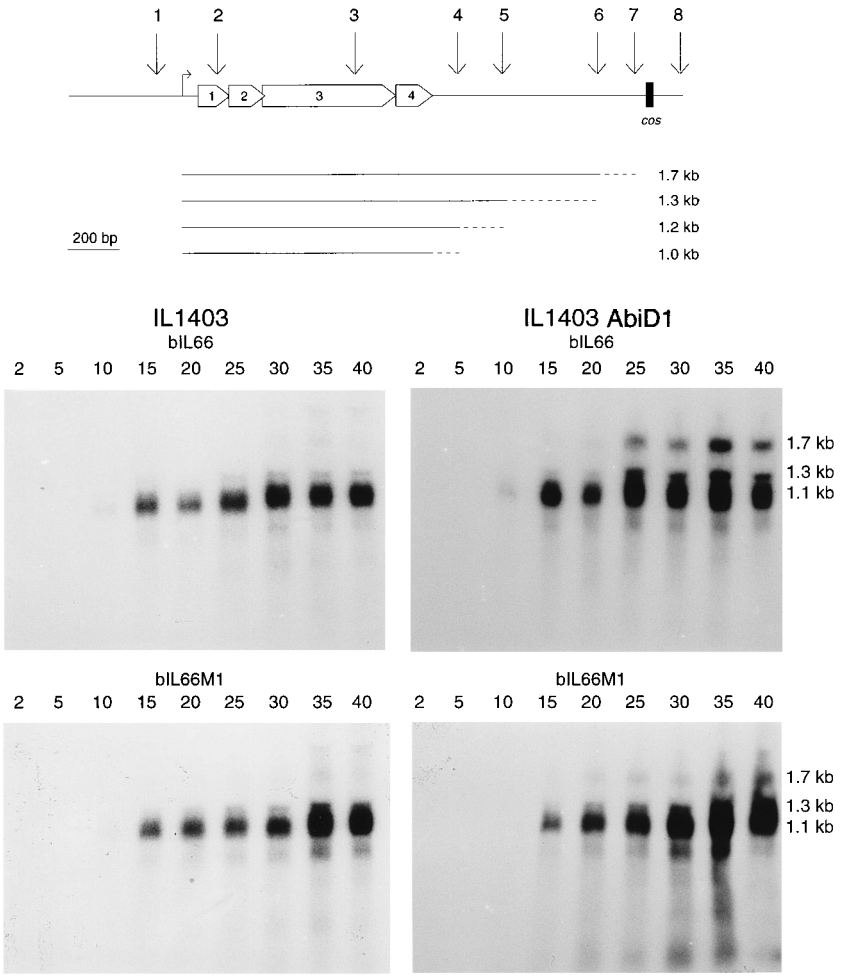

FIG. 4. Northern blot analysis of transcripts specific for the operon formed by *orf1* to *orf4*. RNA extracted at different times after infection of IL1403 or IL1403 AbiD1 with phage bIL66 or phage bIL66M1 was hybridized with oligonucleotide 3, complementary to an internal sequence of *orf3*. The same amount of total cell RNA was loaded in each well. Vertical arrows indicate the positions of oligonucleotides used as probes.

orf1 was identified in each case (Fig. 2). Replacement of G (coordinate 483) or of A (coordinate 501) by a T introduced a stop codon. Replacement of T (coordinate 493) by a C changed a leucine to a proline. Therefore, the resistance of mutant phages to AbiD1 is due either to the absence of *orf1*, as in the case of the nonsense mutation close to the start of the ORF, or a missense mutation within it.

Transcriptional analysis of segment D from phages bIL66 and bIL66M1. A mutation in *orf1* confers partial resistance to AbiD1 (Table 1). This indicates that both *abiD1* and intact *orf1* are needed for the full AbiD1 phenotype. However, the AbiD1 phenotype can be overcome in the presence of an intact *orf1* if *orf3* is expressed in *trans* on a high-copy-number plasmid. This suggests that the level of Orf3 may be limiting for phage growth when *abiD1* and an intact *orf1* are both present. This limitation could result from repression of Orf3 synthesis as well as from Orf3 degradation and/or titration. To discriminate between these alternatives, transcriptional analysis of the *orf3* region was carried out. For this purpose, IL1403 and IL1403 AbiD1 were infected with phage bIL66 or phage bIL66M1, and RNA was extracted at different times after infection and probed with an oligonucleotide complementary to *orf3*. A major band in the 1.1-kb region was detected in all cases 10 to 15 min after infection. It was composed of two transcripts, of 1 and 1.2 kb, which were visible on less-exposed autoradiograms (not shown). Two other transcripts, of 1.3 and 1.7 kb, were also present in all samples. Interestingly, their amount was higher in *abiD1* cells infected with the sensitive wild-type phage than in the other three cases. To map the start and end of the transcripts, eight different oligonucleotide probes complementary to the sequenced region were used (positions are shown in Fig. 4). All transcripts appeared to have the same consensus 5′ end upstream of *orf1* (between oligonucleotides 1 and 2) and different 3′ ends downstream from *orf4* (schematically represented in Fig. 4). A putative RNase E cleavage site (GATTT, 13 bp in front of a stem loop, CCCTGATTCAGGG [10]) is present downstream from *orf4*. The presence of an RNase E equivalent in *L. lactis* has not yet been reported, but processing at this site would generate the abundant 1-kb transcript. The 3′ end of the other transcripts did not correspond either to a terminator structure or to an RNase E processing site, suggesting either a rho-like dependent termination or RNase E-independent processing.

The transcription start was determined precisely by primer extension analysis with transcripts isolated 40 min after infection. The results with bIL66 and bIL66M1 infecting IL1403 are shown in Fig. 5. Identical results were obtained for IL1403 (pIL105) (data not shown). Only one start was detected, at

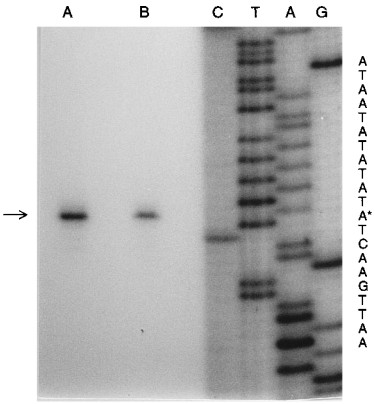

FIG. 5. Primer extension analysis carried out with transcripts from bIL66- (A) and bIL66M1- (B) infected IL1403 cells.

coordinate 454 (Fig. 2). A consensus −10 sequence is present 6 bp upstream of the transcription start, but a −35 consensus sequence is absent. No signal was observed with oligonucleotides corresponding to the putative promoter identified by sequence analysis. Taken together, these results suggest that phage growth is not prevented in AbiD1 cells by repressing transcription of the phage operon.

DISCUSSION

We took advantage of the spontaneous appearance of lactococcal phage bIL66 mutants able to grow on AbiD1 cells to study the molecular mechanisms of phage-AbiD1 interaction. A 1.33-kb DNA segment from mutant phage bIL66M1, when provided in *trans* on a high-copy-number plasmid, allows growth of AbiD1^s phages in AbiD cells. This segment carries an operon composed of four ORFs. Two of these ORFs (*orf1* and *orf3*) were shown to play a role in phage-AbiD1 interaction. Deletion analysis shows that *orf4* has no role in the phenomenon. However, the experiments do not rule out a role for *orf2.*

Intact *orf1* is required for AbiD1 action, since phages lacking it are fully resistant (Table 1). However, expression of *orf3* on a high-copy-number plasmid, even when amputated of its carboxy end, can partially suppress the effect of *abiD1* in the presence of *orf1*. The simplest model to account for these observations is that AbiD1 acts in conjunction with Orf1 to decrease the amount of Orf3 below that required for normal phage development.

A decrease in Orf3 expression does not seem to occur by repressing transcription. The only effect of AbiD1 on transcription of the wild-type phage is to increase somewhat the amount of the largest transcript that covers the operon (Fig. 4). The increase could be due to activation of transcription, antitermination, or inhibition of RNA processing. An argument in favor of the first possibility is that AbiD1 has two helix-turn-helix motifs, found in many DNA-binding proteins (14), and that it therefore activates transcription by binding at the promoter. However, the amounts of other transcripts that originate from the same promoter are not increased, which renders this possibility unlikely. Further work will be required to test the other two possibilities, but the presence of a putative Rnase E site downstream of *orf4* supports the hypothesis that inhibition of RNA processing might account for the increase in the amount of the largest transcript. In this case, AbiD1 in conjunction with Orf1 would have to bind to RNA. It has been reported that RegA, a protein that represses translation of the early mRNA of phage T4 and lacks any known RNA-binding motif, may use a helix-turn-helix motif to bind to RNA (19).

Since the postulated decrease in the amount of Orf3 does not seem to be due to repression of transcription, it could be due to repression of translation of the *orf3* mRNA and/or inactivation of the Orf3 protein. The interaction of AbiD1 with RNA, similar to that discussed above, might affect translation, as does the T4 Reg protein. There are at present no arguments for or against the interaction of Orf3 and AbiD1 at the protein level. Testing of the proposed models is the focus of our future work.

It is of interest that the promoter ensuring transcription of the operon lacks a consensus −35 region. Several such promoters, transcribed by sigma 70, have been described for *E. coli*. They all present an extended −10 promoter sequence consensus (TGNTATAAT), in which the TG motif is an important requirement for efficient initiation (20). Recently, this consensus has been extended (yNtNTGyTATAAT, where y is pyrimidine and N is any nucleotide) (21). The phage bIL66 promoter mapped in this work fits this consensus (TTTGT TATAAT) except for the T at position −19. The position of the transcription start site relative to the −10 box, invariant in −35-independent *E. coli* promoters (21), is conserved in the phage bIL66 promoter. Transcription from *E. coli* −35-independent promoters is as efficient as that of standard σ^{70} promoters and is often modulated by alterations of the −35 region or by activator protein-binding sites (21). This modulation could be advantageous for the delayed expression of the phage operon, which occurs 10 min after infection.

ACKNOWLEDGMENTS

We thank M. Gasson for providing phage phivML3, M. Teuber for providing phage P001, F. Haimet for artwork, and A. Chopin and C. Anagnostopoulos for critical reading of the manuscript.

This work was supported by BRIDGE contract Biot-CT91-0263 from the Commission of the European Communities.

REFERENCES

1. **Anagnostopoulos, C., and J. Spizizen.** 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. **81:**741–746.
2. **Anba, J., E. Bidnenko, A. Hillier, D. Ehrlich, and M.-C. Chopin.** 1995. Characterization of the lactococcal *abiD* gene coding for phage abortive infection. J. Bacteriol. **177:**3818–3823.
3. **Bodorovsky, M., and J. McIninch.** 1993. Genmark: parallel gene recognition for both DNA strands. Comput. Chem. **17:**123–133.
4. **Chandry, P. S., S. C. Moore, B. E. Davidson, and A. J. Hillier.** 1994. Analysis of cos region of the *Lactococcus lactis* bacteriophage sk1. Gene **138:**123–126.
5. **Chopin, A., M.-C. Chopin, A. Moillo-Batt, and P. Langella.** 1984. Two plasmid-determined restriction and modification systems in *Streptococcus lactis*. Plasmid **11:**260–263.
6. **Cluzel, P.-J., A. Chopin, S. D. Ehrlich, and M.-C. Chopin.** 1991. Phage abortive infection mechanism from *Lactococcus lactis* subsp. *lactis*, expression of which is mediated by an iso-IS*1* element. Appl. Environ. Microbiol. **57:**3547–3551.
7. **Coffey, A. G., G. F. Fitzgerald, and C. Daly.** 1991. Cloning and characterization of the determinant for abortive infection of bacteriophage from lactococcal plasmid pCI829. J. Gen. Microbiol. **137:**1355–1362.
8. **Duckworth, D. H., J. Glenn, and D. J. Mccorquodale.** 1981. Inhibition of bacteriophage replication by extrachromosomal genetic elements. Microbiol. Rev. **45:**52–71.
9. **Durmaz, E., D. L. Higgins, and T. R. Klaenhammer.** 1992. Molecular characterization of a second abortive phage resistance gene present in *Lactococcus lactis* subsp. *lactis* ME2. J. Bacteriol. **174:**7463–7469.
10. **Ehretsmann, C. P., A. J. Carpousis, and H. M. Krisch.** 1992. Specificity of *Escherichia coli* endoribonuclease RNase E: *in vivo* and *in vitro* analysis of mutants in a bacteriophage T4 mRNA processing site. Genes Dev. **6:**149–159.
11. **Gautier, M., and M.-C. Chopin.** 1987. Plasmid-determined systems for restriction and modification activity and abortive infection in *Streptococcus cremoris*. Appl. Environ. Microbiol. **53:**923–927.
12. **Gibson, T. J.** 1984. Studies on the Eppstein-Barr virus genome. Ph.D. thesis, Cambridge University, Cambridge, United Kingdom.
13. **Glatron, M. F., and G. Rapoport.** 1972. Biosynthesis of the parasporal

reclusion of *Bacillus thuringiensis*: half-life of its corresponding messenger RNA. Biochimie **54:**1291–1301.
14. **Harrison, S. C.** 1991. A structural taxonomy of DNA-binding domains. Nature (London) **353:**715–719.
15. **Hill, C., L. A. Miller, and T. R. Klaenhammer.** 1990. Nucleotide sequence and distribution of the pTR2030 resistance determinant (*hsp*) which aborts bacteriophage infection in lactococci. Appl. Environ. Microbiol. **56:**2255–2258.
16. **Hill, C., I. J. Massey, and T. R. Klaenhammer.** 1991. Rapid method to characterize lactococcal bacteriophage genomes. Appl. Environ. Microbiol. **57:**283–288.
17. **Holo, H., and I. F. Nes.** 1989. High-frequency transformation, by electroporation, of *Lactococcus lactis* subsp. *cremoris* grown with glycine in osmotically stabilized media. Appl. Environ. Microbiol. **55:**3119–3123.
18. **Jarvis, A. W., G. F. Fitzgerald, M. Mata, A. Mercenier, H. Neve, I. B. Powell, C. Ronda, M. Saxelin, and M. Teuber.** 1991. Species and type phages of lactococcal bacteriophages. Intervirology **32:**2–9.
19. **Jozwik, C. E., and E. S. Miller.** 1992. Regions of bacteriophage T4 and RB69 RegA translational repressor proteins that determine RNA-binding specificity. Proc. Natl. Acad. Sci. USA **89:**5053–5057.
20. **Keilty, S., and M. Rosenberg.** 1987. Constitutive function of a positively regulated promoter reveals new sequences essential for activity. J. Biol. Chem. **262:**6389–6395.
21. **Kumar, A., R. A. Malloch, N. Fujita, D. A. Smillie, A. Ishihama, and R. S. Hayward.** 1993. The minus 35-recognition region of *Escherichia coli* sigma 70 is inessential for initiation of transcription at an "extended minus 10" promoter. J. Mol. Biol. **232:**406–418.
22. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Moineau, S. E., E. Durmaz, S. Pandian, and T. R. Klaenhammer.** 1993. Differentiation of two abortive mechanisms using monoclonal antibodies directed toward capsid proteins. Appl. Environ. Microbiol. **59:**208–212.
24. **Saraste, M., P. R. Sibbald, and A. Wittinghofer.** 1990. The p-loop—a common motif in ATP- and GTP-binding proteins. Trends Biochem. Sci. **15:**430–434.
25. **Schouler, C., S. D. Ehrlich, and M.-C. Chopin.** Sequence and organization of the lactococcal prolate-headed bIL67 phage genome. Microbiology **140:** 3061–3069.
26. **Shearman, C., H. Underwood, K. Jury, and M. Gasson.** 1989. Cloning and DNA sequence analysis of a *Lactococcus* bacteriophage lysin gene. Mol. Gen. Genet. **218:**214–221.
27. **Simon, D., and A. Chopin.** 1988. Construction of a vector plasmid family for molecular cloning in *Streptococcus lactis*. Biochimie **70:**559–566.
28. **Snyder, L.** 1995. Phage-exclusion enzymes: a bonanza of biochemical cell biology reagents? Mol. Microbiol. **15:**415–420.
29. **Tanaka, T.** 1979. Restriction of plasmid-mediated transformation in *Bacillus subtilis* 168. Mol. Gen. Genet. **175:**235–237.
30. **Terzaghi, B. E., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Environ. Microbiol. **29:**807–813.
31. **Van de Guchte, M., J. Kok, and G. Venema.** 1992. Gene expression in *Lactococcus lactis*. FEMS Microbiol. Rev. **88:**73–92.
32. **Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay.** 1982. Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. **1:**945–951.
33. **Williams, J. G., and P. J. Mason.** 1985. Hybridization in the analysis of RNA, p. 139–160. *In* B. D. Hames and S. J. Higgins (ed.), Nucleic acid hybridization—a practical approach. IRL Press, Oxford.